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Toward A variable RBE for proton beam therapy

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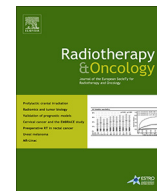
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Review

Toward A variable RBE for proton beam therapy

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ABSTRACT

In the clinic, proton beam therapy (PBT) is based on the use of a generic relative biological effectiveness (RBE) of 1.1 compared to photons in human cancers and normal tissues. However, the experimental basis for this RBE lacks any significant number of representative tumor models and clinically relevant endpoints for dose-limiting organs at risk. It is now increasingly appreciated that much of the variations of treatment responses in cancers are due to inter-tumoral genomic heterogeneity. Indeed, recently it has been shown that defects in certain DNA repair pathways, which are found in subsets of many cancers, are associated with a RBE increase *in vitro*. However, there currently exist little *in vivo* or clinical data that confirm the existence of similarly increased RBE values in human cancers. Furthermore, evidence for variable RBE values for normal tissue toxicity has been sparse and conflicting to date. If we could predict variable RBE values in patients, we would be able to optimally use and personalize PBT. For example, predictive tumor biomarkers may facilitate selection of patients with proton-sensitive cancers previously ineligible for PBT. Dose de-escalation may be possible to reduce normal tissue toxicity, especially in pediatric patients. Knowledge of increased tumor RBE may allow us to develop biologically optimized therapies to enhance local control while RBE biomarkers for normal tissues could lead to a better understanding and prevention of unusual PBT-associated toxicity. Here, we will review experimental data on the repair of proton damage to DNA that impact both RBE values and biophysical modeling to predict RBE variations. Experimental approaches for studying proton sensitivity *in vitro* and *in vivo* will be reviewed as well and recent clinical findings discussed. Ultimately, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limit treatment toxicity should fundamentally impact the clinical use of PBT.

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Introduction

There exists a great potential for therapeutic benefits of proton beam therapy (PBT) in several cancer types [1]. PBT has superior physical characteristics compared to standard photon radiation in many anatomical sites, but its biological properties have been thought to be similar to photons [2,3]. This is reflected by the use of a generic relative biological effectiveness (RBE) of 1.1 for both cancer and normal tissues (Fig. 1). However, there exists a scarcity of data on RBE variations in human tumors. In a comprehensive review from 2002, the average RBE was estimated as ~1.2 *in vitro* and ~1.1 *in vivo*. However, most of the 20 cell lines

were of Chinese hamster ovary (CHO) origin resulting in a somewhat higher *in vitro* RBE, and only 7 human cancer cell lines were included [2]. In a recent update, the number of cancer cell lines remained limited, with an average RBE of ~1.15 [3]. However, there remains considerable variability related to both experimental conditions (incl. dose, beam characteristics) and cell biology (incl. DNA repair status, α/β ratio).

This experimental basis for the current clinical use of a generic RBE of 1.1 is a major limitation, given the considerable genomic heterogeneity across cancers even for the same type and histology as unraveled by recent genomic studies. Moreover, it is increasingly appreciated that much of the variations in treatment sensitivity observed clinically are due to inter-tumoral heterogeneity, which includes alterations in the DNA damage response (DDR) [4–9]. Indeed, several reports have now demonstrated that defects

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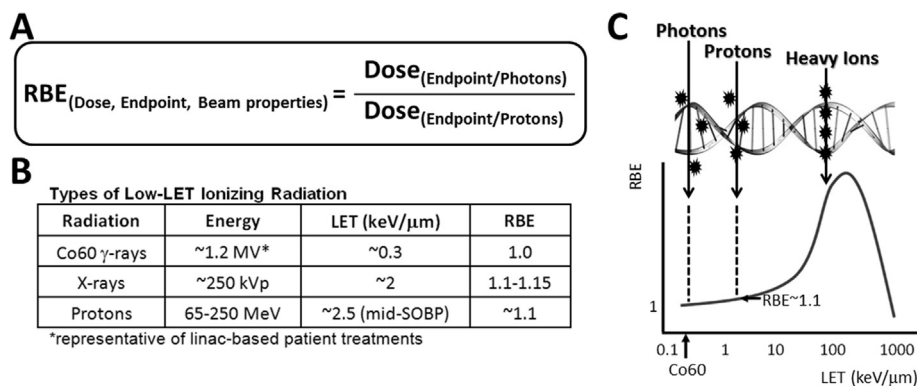


Fig. 1. Relative Biological Effectiveness (RBE) of different ionizing radiation qualities. (A) RBE is the ratio of doses to reach the same level of effect (Endpoint) when comparing two radiation modalities, i.e., reference photons (Cobalt-60, Co60) vs. protons. The radiation dose in PBT is prescribed as Gy[RBE] according to ICRU. For example, for a RBE = 1.1 and a desired Co60 equivalent (Co60Eq) dose of 2 Gy, the corresponding physical proton dose would be 1.82 Gy (1.82 Gy \times 1.1 = 2 Gy[RBE]). (B) Examples of ionizing radiation types and representative dose-average LET (Linear Energy Transfer) and RBE values [10,103,104]. LET of γ-rays refers to secondary electrons. (C) Illustration of how protons induce DNA damage that is slightly more clustered than photons (or Co60 γ-rays), which in DNA repair-proficient cells yields a RBE of 1.1.

in the homologous recombination (HR) and Fanconi Anemia (FA) DDR pathways are associated with RBE values of 1.3 or more *in vitro* [10–13]. However, there currently exist few pre-clinical *in vivo* or clinical data to demonstrate the existence of increased RBE values in human cancers, and evidence with regard to RBE variations in normal tissues remains sparse as well [2,14–16].

Importantly, we lack an in-depth understanding of the mechanisms that underlie RBE variations in tumors and normal tissues, and we are currently unable to identify individual cancer patients whose tumors and/or normal tissues exhibit increased sensitivity to PBT. These shortcomings constitute critical barriers to fully harnessing the potential superiority of PBT and to avoiding unnecessary toxicity. Here, we review our current knowledge of and approaches to understanding RBE variations in tumors and normal tissues. In the not too distant future, therapeutically exploiting the understudied biological advantages of protons and developing approaches to limiting treatment toxicity are expected to fundamentally impact the clinical use of PBT in the increasing number of proton centers worldwide.

DNA damage caused by particle radiation and its repair

DNA repair of double-strand breaks and clustered damages

Although the use of charged particle therapy has increased rapidly over the last few decades, the contribution and the interplay of specific DNA repair pathways to the repair of DNA lesions induced by these radiation modalities is incompletely understood. Particle radiations such as proton or carbon ion beams induce more highly localized and clustered DNA damage than X- and γ-rays. Clustered DNA damage includes abasic sites, base damages, single- (SSBs) and double-strand breaks (DSBs) that are in close proximity to each other [17]. The complexity and yield of radiation-induced clustered DNA damage increases with ionization density [18–21]. Hence, for a given dose, therapeutic carbon beams (200–430 MeV/n; \sim 10–80 keV/μm linear energy transfer (LET)) are expected to induce more clustered DNA lesions than therapeutic proton beams (65–250 MeV/n; \sim 2–10 keV/μm). Clustered DNA damage represents a considerable obstacle to efficient repair, and DSBs within clustered lesions rejoin with slower kinetics and less completely than frank DSBs [20,22], likely contributing to the observed higher RBEs for cell killing after charged particle- compared to photon-irradiated cells [18,23]. A major question is whether the mechanisms of repairing DNA damages caused by PBT resemble those triggered by photons or those operating in response to heavy ion exposure.

When potentially lethal DSBs occur, cells repair these DNA ends mainly by two distinct pathways, non-homologous end joining (NHEJ) and HR. These two pathways differ biochemically, have different substrate requirements, and are used differently throughout the cell cycle (for review, see [24]). Briefly, NHEJ is the main pathway of ionizing radiation-induced DSB repair in G1- and early S-phase cells while both HR and NHEJ contribute to DSB repair in late S-/G2-phase cells [25,26]. Importantly, HR also is the predominant pathway for the repair of stalled and damaged DNA replication forks [27,28]. Notably, mutations in HR genes increase cellular sensitivity to photon radiation and also to replicative and transcriptional stress [29,30]. During HR a DSB, or a DNA replication fork encountering a DNA lesion, undergoes nucleolytic resection to yield 3' single-stranded (ss) DNA ends which are immediately covered by the ssDNA binding protein replication protein A (RPA). RPA is then replaced by the RAD51 recombinase forming a nucleoprotein complex termed the presynaptic filament. The presynaptic filament searches for, engages, and invades a homologous duplex target DNA to form the displacement loop (D-loop). DNA synthesis and resolution of DNA intermediates follows to complete HR repair [31]. During NHEJ, the KU70/80 heterodimer, which has high affinity for free DNA ends [13], initiates the pathway, whereby nucleolytic processing of DNA ends is blocked. KU70/80 recruit DNA-PKcs, and DNA-PKcs immobilizes the two DNA ends and facilitates the rejoining reaction [32–34], in which ligation is carried out by the XRCC4-DNA ligase IV complex [35]. NHEJ is the major repair pathway for DSBs induced by photon radiation including X-rays (for review, see [36]).

Repair pathways for high-LET radiations

To date, only a limited number of studies have addressed the relative contributions of NHEJ, HR and resection-mediated repair pathways to removing complex DSBs induced by different charged particle radiation types. Evidence is accumulating that shows that NHEJ is less capable of removing clustered DSBs induced by high-LET radiations as compared to low-LET radiations [37–42]. Yajima *et al.* [42] investigated the propensity of human and mouse cells to undergo DNA resection after low-LET (X- or γ-rays) versus high-LET radiations (70 keV/μm carbon (290 MeV/n) or 250 keV/μm iron ions (500 MeV/n)). Their study showed that >80% of the DSBs induced by heavy ions were subjected to end resection, which is significantly more than what was observed after low-LET radiations [41,43]. Interestingly, Yajima *et al.* [42] also reported on DNA resection occurring in G1 phase cells after heavy ion treatment and suggested that microhomology-mediated end-joining

(MMEJ) may take over from inefficiently working NHEJ at clustered DSBs in G1-phase cells. These findings are in accord with results reported by Averbeck and collaborators [44], who investigated G1-phase cells and a large number of heavy ion beams including 90 and 170 keV/ μm carbon ions. These investigators also concluded that an increased requirement for processing of DNA ends at complex DSBs forces DNA repair pathway choice in G1-phase cells toward resection-dependent MMEJ repair [44].

One of the first studies that directly investigated the involvement of the HR pathway in repairing complex DSBs induced by heavy ions (1 GeV/n iron ions; LET = 150 keV/ μm) compared the response to iron ions of HR-proficient and deficient Chinese hamster ovary (CHO) cell lines, and of syngeneic human cells depleted for the key HR protein RAD51 or its paralog RAD51D by RNA interference [45]. HR capability after iron ion irradiation was found to contribute to cell survival in rodent cells and to limiting mutagenesis in human cells, whereby the requirement for an intact HR pathway in protecting cells from the detrimental effects of charged particle radiation was demonstrated [45]. Interestingly, when the relative contributions of the NHEJ and HR pathways to the repair of DNA lesions induced by 200 MeV protons (energy spread at the mid-SOBP = 0–60 MeV; dose-average LET = 2.2 keV/ μm), 290 MeV/n carbon ions (energy spread at the mid-SOBP = 0–160 MeV; dose-average LET = 50 keV/ μm) or γ -rays were compared in CHO cell lines, HR capability was found to be more critical for the repair of carbon ion-induced DSBs than for the repair of DSBs induced by protons or by γ -rays [46].

The effects of a range of different heavy ions, including a 290 MeV/n carbon ion SOBP beam (LET = 50 keV/ μm), on the survival of mouse embryonic fibroblasts (MEFs), fully DSB repair proficient or compromised in NHEJ or HR by homozygous deletion of the *Lig4* or *Rad54* genes, respectively, showed that defects in NHEJ lead to higher cellular sensitivity to therapeutic carbon beams than defects in HR [47]. However, it is interesting to note that, when compared to 200 kVp X-rays, RBE values for SOBP carbon ions at 10% survival were actually higher for *Rad54*-deficient MEFs (~ 2.1) than for *Lig4*-deficient MEFs (~ 1.4) [47]. These data suggest that, similar to the findings by Gerelchuluun *et al.* [46], even though NHEJ-deficiency is linked to the greatest cytotoxicity for all radiation types tested, exposure of HR-deficient cells to carbon ions is still more effective in reducing cell survival than exposure of HR-deficient cells to X-rays.

Repair pathways for low-LET protons

Grosse *et al.* reported that the removal of proton-induced DSBs preferentially relied on HR when comparing the cytotoxicity of 200 kVp X-rays to protons (energy spread at the mid SOBP ≤ 138 MeV) in CHO cells [13]. This study also showed that the DSB repair kinetics in HR-deficient cell lines were significantly delayed after proton irradiation compared to X-rays, and the authors concluded that PBT may be particularly beneficial to cancer patients with mutations in HR pathway genes. In a follow-up study, Fontana and collaborators investigated NHEJ and HR pathway choice in human A549 lung cancer cells depleted for RAD51 and in BRCA2-deficient ovarian carcinoma cells after exposure to X-rays and protons [12]. In accord with the results from their earlier study [13], an enhanced susceptibility of HR-deficient tumor cells to protons and an increased sensitivity of photon-irradiated tumor cells to NHEJ inhibitors were detected [12], further supporting the notion that tumors with defects in HR may be more susceptible to PBT than to photons. Similarly, data based on monitoring RAD51 and 53BP1 foci formation by stimulated emission depletion (STED) microscopy in S/G2-phase HeLa cells exposed to 21 MeV protons (LET = 2.56 keV/ μm) showed that the HR pathway may be operating at higher levels after protons [48] than after photons [41].

An open question remained as to whether HR repair of DSB in late S/G2-phase or DNA replication fork-associated HR was required for the repair of proton-induced DNA damage. In a screen of 17 human lung cancer cell lines exposed to a clinical proton beam at mid-SOBP (235 MeV; 2.5 keV/ μm), a range of proton RBE values was observed. Increased RBE values in the lung cancer and other human cell lines correlated with defects in HR or FA genes [49]. Importantly, HR and the FA pathway cooperate specifically at DNA replication forks to ensure fork protection and restart [28,50]. These data, therefore, suggested that clustered DNA damage resulting from proton irradiation challenges the progression of DNA replication forks in S-phase cells, leading to increased dependency on the HR/FA pathways for DNA damage repair and replication fork restart [49,51]. Liu and coworkers [11] corroborated this presumption demonstrating that immortalized human fibroblasts derived from a FA complementation group P patient with biallelic *SLX4* mutations are hypersensitive to protons, and that this hypersensitivity was linked to the *SLX4*-interacting MUS81 protein. Notably, both *SLX4* and *MUS81* are required for replication fork restart and HR (for review, see [28]).

In contrast to these studies [10–13], the report by Gerelchuluun *et al.* did not find an increased RBE of protons compared to X-rays when HR was impaired due to loss of XRCC2 or XRCC3 function [46]. The reason for this discrepancy remains unclear but may relate to assay parameters. Gerelchuluun *et al.* employed immediate plating for colony formation after irradiation which was not used in the other studies. It is possible that early trypsinization interferes with cell cycle progression and reduces the odds of DNA replication forks encountering unrepaired DSBs. Taken together, the majority of the published data suggest that the differential DNA repair capabilities between tumor and surrounding normal tissues could be exploited to improve regimens of PBT.

Since genetic or epigenetic alterations in HR and FA genes are increasingly being detected in many different cancer types [52–54], more cancer patients than historically thought may be appropriate candidates for PBT. Conceivably, other DDR defects and tumor genotypes may also be associated with increased RBE values. More *in vivo* and clinical data are urgently needed to substantiate the promising data obtained in cell culture experiments. It will also be necessary to more systematically investigate the link between HR/FA repair deficiencies and carbon ions, to improve our understanding of the similarities and differences of clustered DNA lesions induced by protons vs carbon ions. Ideally, a larger set of genomically characterized human cancer cell lines should be included in these experiments, as it has been done for protons in the past [49]. A model to depict the effects of different radiation qualities combined with deficiencies in the HR/FA pathways on RBE values is shown in Fig. 2.

Modeling and predicting the RBE of proton beam therapy

Even if we could determine the effects of DNA repair deficiencies that influence proton sensitivity in tumors, this may not be enough to predict clinically relevant RBE variations. Such predictions require quantitative models to assess how physical factors, such as radiation dose and LET, and biological parameters, such as tumor type and functional DNA repair status, combine within an individual's treatment. However, despite the growing understanding of the mechanistic drivers of RBE as described above and elsewhere [3], few biophysical models fully incorporate this knowledge, but instead fall into two broad categories.

Firstly, a number of models use conventional linear-quadratic dose responses, and modify the α and β parameters with LET-dependent terms to incorporate increasing damage complexity [55–57]. A second group of models incorporate increased physical

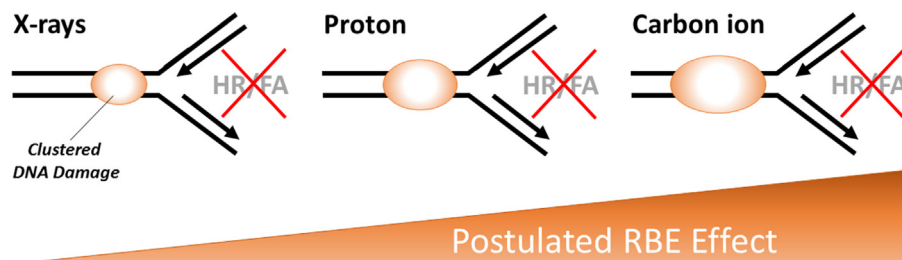


Fig. 2. Model: HR/FA defects lead to an increase in RBE as a function of clustered damage complexity. Cells with defects in DNA repair by homologous recombination (HR) or the Fanconi Anemia (FA) pathway are impaired in the ability to repair DNA replication forks that encounter clustered DNA damages. Even though the average LET is similar between orthovoltage X-rays and protons at mid-SOBP, protons are hypothesized to generate more complex lesions that present a greater obstacle to HR/FA-deficient cells than those produced by X-rays [10]. Clustered damages after high-LET carbon ion beam irradiation likely rely on an intact HR pathway for removal as well.

detail by replacing averaged LET with detailed sub-cellular dose calculations, including the Microdosimetric Kinetic Model [58], Local Effect Model [59], and Monte Carlo Damage Simulation [60]. A common feature of these models is that they do not incorporate significant detail about the underlying biology, typically extrapolating empirical parameters by fitting between photon and proton exposures. As a result, they have limited ability to incorporate our knowledge of the impact of genomic heterogeneity, in particular aspects of genomically determined radiation sensitivity. Consequently, new models are needed if inter-patient RBE heterogeneity is to be successfully incorporated into treatment planning.

Numerous models of radiation response processes are under development, including biophysical models of radiation-induced DNA damage distribution and complexity, models of DSB repair incorporating kinetics of protein recruitment and damage complexity, and models of cell death following different stresses [61–67]. But while these models can provide useful insights, most focus on a single endpoint, making them unsuitable to fully describe the variations in RBE which depend on multiple physical and biological factors. Integrated multi-scale models are needed to enable us to incorporate our knowledge of these processes to deliver individualized RBE predictions.

Some groups have begun to develop links between these different scales, including recent developments in the Local Effect Model, Giant Loop Binary Lesion Model, PARTRAC, and others [68–73]. These models have helped to compare which factors may be most significant in driving variations in RBE, and they have demonstrated the benefits of combining these factors into integrated models. However, the development of such models is challenging as each additional process to be modeled requires additional parameters which must be characterized and quantified, introducing uncertainties which are impossible to address through studies of cell survival based RBE values alone.

Development of multi-scale models instead demands close collaboration between experimentalists and modelers to design multi-scale experiments, combining terminal endpoints such as survival with quantification of intermediate processes such as DNA repair and other endpoints such as mutation or chromosome aberration formation to allow all stages of cellular radiation response to be characterized. By integrating all aspects of radiation response in individual studies, inter-experimental uncertainties can be reduced, enabling the development of more robust and extensible individualized RBE models.

Experimental approaches for determining proton RBE *in vitro* and *in vivo*

Although the number of operating proton therapy centers worldwide has been increased to more than 60, with currently 26 in the United States (www.ptcog.ch), it is important to realize

that access to those centers for biological *in vitro* and *in vivo* experiments will likely be limited. This is not reflecting a lack of interest in proton research but rather the economical need for a financial return of an expensive treatment facility as well as the paucity of suitable laboratory space and animal facilities close by.

At the same time, there is a great need for more comprehensive and systematic RBE studies. The increased RBE in the distal fall-off of the Bragg-peak holds the potential for risks, i.e. potentially increased side effects due to uncertain RBE, as well as benefits, i.e. potentially enhanced tumor local control rates if they can be exploited through the use of intensity-modulated proton beam therapy. Additionally, RBE values in some tumors may be increased due to genomic factors as discussed above. Treatment planners will tackle dose distributions and range uncertainties, but pre-clinical research will need to shed light onto proton-specific normal tissue complications and tumor sensitivities. These findings should support patient stratification and guide a clinician's decision whether or not PBT is favored in an individual patient. Therefore, a proton-specific experimental pipeline is needed to support the translation of pre-clinical findings into clinically useful knowledge.

Recently, a pipeline for pre-clinical studies of radiation/drug combinations was proposed, emphasizing the importance of the *in vitro* clonogenic survival assay, medium/high-throughput assays for drug screening, physiological 3-dimensional (3D) assays as well as growth delay and tumor control experiments *in vivo* [74]. All of these techniques and endpoints have similar relevance for proton research. Limited access to proton beam time, nonetheless, lowers the number of biological samples which can be exposed and directly influences the range of parameters that can be studied in combination with proton radiation, e.g. number of cell lines/models, time points, radiation doses, drugs in different concentrations. As a consequence, the question arises which biological models should be used for proton research. It is increasingly held that a small number of established cell lines are not representative of the heterogeneity of human cancers although controversy exists [75–77]. Established cell lines do have the advantage that they grow reliably, are often genomically characterized, and are functionally studied (including characterization of radiation sensitivity). Over 20 years ago, the NCI60 cell line panel comprising nine cancer entities was established to standardize cancer drug research. Similar platforms are being developed for radiation purposes [78,79]. Analogously, panels of annotated cancer and normal cell lines with clinical relevance for PBT would be very useful. Such cell lines should have known X-ray (+/– drug) sensitivities. Selective inclusion of responder and non-responder cell lines for proton experiments would be of great value for mechanistical insight and for translational approaches as they might be indicative for clinical patient selection. Primary cell cultures preferentially grown as 3D models would be useful to confirm findings. Especially patient-derived cultures such as tumor or normal tissue organoids could help to stratify patients in the future if a correlation of results

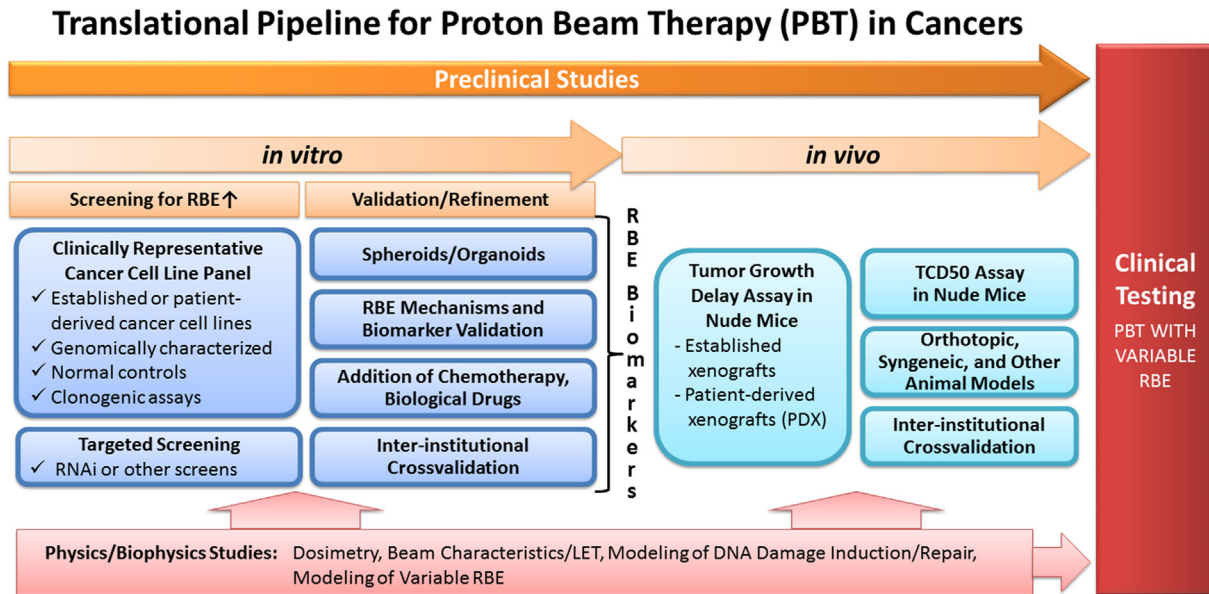


Fig. 3. Proposed translational pipeline for discovering clinically useful biomarkers that predict variable RBE values. See text for details.

from *in vitro* organoid cultures and patients' treatment outcomes could be established.

Performing *in vivo* experiments at clinical or experimental proton beam lines poses further challenges, including positioning of the animal and dose description for a small field [80]. For normal tissue endpoints, the RBE of protons is generally in the order of ~1.1 as reviewed [2,81]. However, much of these data pertain to skin reactions, and clinically significant endpoints such as involving the central nervous system or heart remain poorly studied. For tumor models, there exists a paucity of data. The tumor growth delay for a subcutaneous FaDu xenograft model following pulsed and continuous proton beams in comparison to 6 MV photons led to RBE values of 1.22 and 1.10, respectively [82]. In a Hep3B hepatoma xenograft model treated with 3 daily doses of 3 Gy 230 MeV protons at mid-SOBP or 6 MV photons, proton treatment resulted in 1.4-fold greater tumor growth delay which was statistically significant. Moreover, the *in vivo* effect mirrored the increased proton sensitivity seen in an *in vitro* colony formation assay, where the RBE at 0.5 survival fraction was around 1.5 (estimated from Fig. 2c in Ref. [82]). The authors also demonstrated that the difference in tumor effect between protons and photons could be pharmacologically enhanced. More *in vivo* data are urgently needed to derive *in vivo* RBE estimates and identify molecular targets for proton-specific radiosensitization.

Experience in photon research highlights the enormous relevance of tumor xenograft models for clinical translation [83–85]. Particularly, local tumor control dose experiments have demonstrated great potential relative to tumor growth delay in translating knowledge to the clinic [86,87]. However, subcutaneously transplanted tumors reach the desired tumor volume with temporal variations. Given the most often strictly defined days of proton beam time, loss of a large number of animals due to tumors of undesirable size faces ethical and biostatistical concerns. Furthermore, clinically relevant fractionation schedules, such as 30 fractions in 6 weeks, need to be adapted to proton beam availability. Here, shorter, hypofractionated schedules may be a viable and clinically relevant option. Still, local tumor control (TCD₅₀) experiments require approximately 80–100 animals per treatment arm, which essentially limits these studies to proton centers with dedicated rooms for experimental purposes.

To conclude, the establishment of a proton-adapted NCI60-like cancer cell line panel would be desirable to study mechanistic RBE effects, especially in combination with targeted drugs (Fig. 3). Patient-derived 3D/organoid cultures should be used for validation of results and, if a correlation with patient-outcome can be shown, may guide patient stratifications in the future. Clinically relevant *in vivo* experiments will be the bottle neck for the translational chain. These studies should consider not only relevant tumor models but also normal tissue endpoints, LET, and fractionation and thus may be best performed in centers with dedicated experimental rooms.

Toward clinical evidence for a variable proton RBE

In the clinical practice of heavy ion beam therapy, biologic effect models are explicitly considered in the treatment planning process in order to limit high-LET depositions in critical normal tissues. While the models used for treatment planning may differ between centers, with different models being used in Asia and Europe, variable RBE is incorporated in each. This has not been the case for PBT where it has been assumed that the RBE of protons is a constant value of 1.1 [2]. Around the world there has been a rapid expansion in the number of proton centers. As the number of patients treated with PBT increases, there is growing debate regarding the RBE of PBT [51,88–91].

In the laboratory, recent evidence indicates that the capacity for proton beams to cause biological damage is substantially higher near the distal, high-LET region [81,92–94]. This is true both for tumors as well as normal tissues. This is, of course, well known for heavy ions, but as yet not explicitly incorporated into proton treatment planning. Arguments most often made in support of a continued use of an RBE of 1.1 include that relative to heavy ions, the areas of high LET within a proton beam are very small and likely clinically insignificant if not within a critical organ at risk, and that there are no clinical data to suggest that the proton RBE is not 1.1 in most patients.

Increasingly, however, investigators are challenging the use of a generic RBE value of 1.1 for PBT using laboratory and clinical data. There are now several studies which have documented increased cell kill in the distal regions of proton beams, which

is supported by the observation of differential DNA damage along the beam path [92,93]. Clinically, the group at MD Anderson documented increased rates of post-radiation MR imaging changes in ependymoma patients treated with PBT compared to photons [14]. Such changes are indicative of early radiation injury and serve as an imaging biomarker of differential damage between these radiation types. In order to further evaluate the etiology of these changes, this group used Monte Carlo techniques to compute proton dose and LET distributions and found significant correlations between LET, dose, and regions of imaging change in these patients. Others are now expanding research in this area by conducting laboratory investigations, using normal tissue models, and clinical studies in expanded patient cohorts and other disease sites to better characterize the biologic effectiveness of PBT. Mining existing data bases and registries, especially at high-volume proton centers, may reveal associations between unusual biological effects and PBT (https://www.ptcog.ch/archive/patient_statistics/Patientstatistics-updateDec2016.pdf). For example, recent patient data indicate that for parenchymal lung changes induced by end-of-range protons the RBE is >1.1 [95]. On the other hand, no apparent increase was observed in brainstem necrosis in pediatric patients treated with PBT [96]. In contrast to a potentially variable tumor RBE, biological mechanisms underlying any RBE variations in normal tissues (other than low α/β values and LET increases at end-of-range) are essentially unknown at this time.

High level evidence for an increase in tumor RBE >1.1 in patients treated with PBT is also currently lacking. Because RBE increases may only exist in a subset of patients, such as the 20–25% of patients with HR/FA defects [97], associated local tumor control or survival effects are most likely missed when analyzing the study populations as a whole. Ideally, predictive biomarkers for tumor RBE >1.1 are needed to enrich a study population before randomizing patients to PBT vs photons in a clinical trial. Searches for a clinical signal of increased proton sensitivity and unusual responders in the randomized trials of PBT vs photons completed or soon to be completed will be of great interest [98] (NCT01512589, NCT01893307).

As the amount of data regarding a role for variable RBE increases, it seems that the field of PBT may follow that of heavy ions, with assessments of biological effectiveness incorporated into the treatment planning process. With newer proton therapy delivery modalities, in particular spot scanning proton therapy, intensity modulated proton therapy (IMPT) plans may be developed to preferentially divert high LET protons away from normal tissues into the target volume. This should reduce normal tissue damage while simultaneously increasing biologically effective doses to targets and perhaps even augmenting pre-existing RBE advantages due to certain DNA repair defects, further expanding the therapeutic index for PBT.

Conclusions

If we could predict variable RBE values in patients, we would be able to optimally use and personalize PBT. For example, such markers may facilitate selection of patients with proton-sensitive cancers if these patients would have been otherwise ineligible for PBT. Dose de-escalation may be possible to reduce normal tissue toxicity especially in pediatric patients. Knowledge of an increased tumor RBE may allow us to develop biologically optimized therapies to enhance local control, for example through optimized IMPT or specific drug combinations, while RBE biomarkers in normal tissues could lead to a better understanding and prevention of unusual PBT-associated toxicity. We also note that there continues to be a major international debate regarding the cost-effectiveness

of proton therapy [1,99–102]. Public, policy makers, and payers want to see evidence for the superiority of PBT and quantification of benefit, which to date has been framed only in technological terms. Realizing a biological advantage of PBT in subsets of cancer patients (and/or an increased risk for treatment toxicity in some patients), would fundamentally impact and redirect this debate.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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